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13. ABSTRACT ***(Maximum 200 Words)***

This study tests whether breast cancer can be eliminated by immunization with foreign peptides followed by delivery of peptides to tumors. We proposed to (1) establish an in vitro assay to measure tumor growth inhibition, (2) synthesize pro-peptides for activation at the tumor site by beta-glucuronidase, and (3) test tumor rejection with peptide therapy. In this funding year, Flu MP58 pro-peptide was synthesized, purified to 76% purity with preparative HPLC and was suitable for HLA binding analysis. MP58 peptide bound to human HLA-A2.1 cells in a dose dependent manner in the range of 15 to 60 μM. MP58 pro-peptide up to 120 μM did not bind to HLA-A2.1. In the presence of 200 or 300 unit/ml of beta-glucuronidase, A2.1 binding MP58 was released from the pro-peptide as demonstrated by its binding to HLA-A2.1. The synthesis and purification of MP58 pro-peptide demonstrated the chemical feasibility of pro-peptide development. The release of active peptide by beta-glucuronidase demonstrated the precise and controlled generation of an antigenic epitope from a stealth agent as predicted by our design. Pro-peptide is a new and novel agent with strong potential for cancer immunotherapy.

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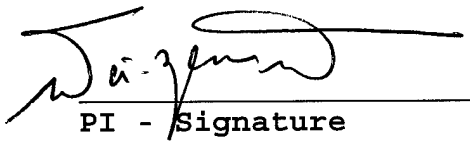

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INTRODUCTION

This project continues to test the concept of developing pro-peptides as anti-cancer therapeutic agents. The proposed tasks are the following.

Task 1 Establish an in vitro assay to measure tumor growth inhibition by peptide specific T cells and pro-peptides

Task 2 Synthesize and test the activity of pro-peptides to be activated by β-glucuronidase at the tumor site

(A) Synthesis of glucuronide derivatives of Flu peptide MP-58 GILGFVFTL presented by human HLA-A2.1.

(B) Measurement of peptide and pro-peptide binding to MHC.

Task 3 Test tumor rejection in mice immunized with the foreign peptides and treated with the pro-peptides.

(A) Measurement of β-glucuronidase activity in mouse mammary tumors.

(B) Measurement of reactive T cell frequency in peptide immunized mice.

(C) Measurement of pro-peptide and peptide distribution in vivo.

(D) Measurement of tumor growth inhibition in mice.

BODY

Task 1 Establish an in vitro assay to measure tumor growth inhibition by peptide specific T cells and pro-peptides

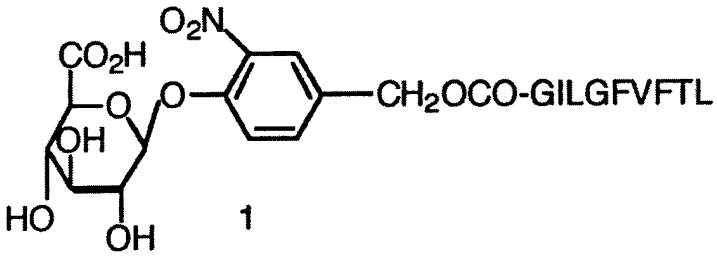
Results from this task were described in the last progress report and will not be repeated here. The primary effort during the funding period of July 1, 2000 to June 30, 2001 was focused on the synthesis and testing of MP58 pro-peptide.

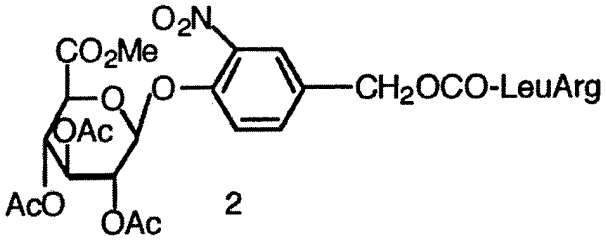
Task 2 Synthesize and test the activity of pro-peptides to be activated by β-glucuronidase at the tumor site

(A) Synthesis of glucuronide derivatives of β-gal peptide TPHPARIGL.

As described in the last report, Flu peptide MP-58 GILGFVFTL presented by human HLA-A2.1 rather than beta-galatosidase peptide TPHPARIGL presented by mouse K^d was used for pro-peptide development because the residues are less reactive and there is potential human application. A synthetic approach to antigenic prodrug of GILGFVFTL was as previously described (appendix). Column chromatographic methods failed to give material of sufficient purity for biological testing. Therefore, octapeptide ILGFVFTL, a starting material for synthesis of 1, was purified by preparative HPLC and it was obtained in 91% purity. The resultant prodrug was purified in a similar fashion at 76% purity. This material was used for MHC I binding and cytotoxic T cell analysis. Currently, compound 1 is being prepared on a larger-scale to provide sufficient material for more extensive biological testing.

Previously, we reported that "prodrug" of a model peptide LeuArg has the glucuronic portion attached to a guanidino group of arginine. However, ^{1}H-NMR spectra and negative reaction with ninhydrin clearly indicated an attachment at the N-terminus of leucine (structure 2). This finding has relevance for synthesis of antigenic β-galactosidase nonapeptide p876 TPHPARIGL which contains arginine. It is likely that a synthetic strategy similar to that used for prodrug 1 will be applicable for introducing the same prodrug moiety into this nonapeptide.



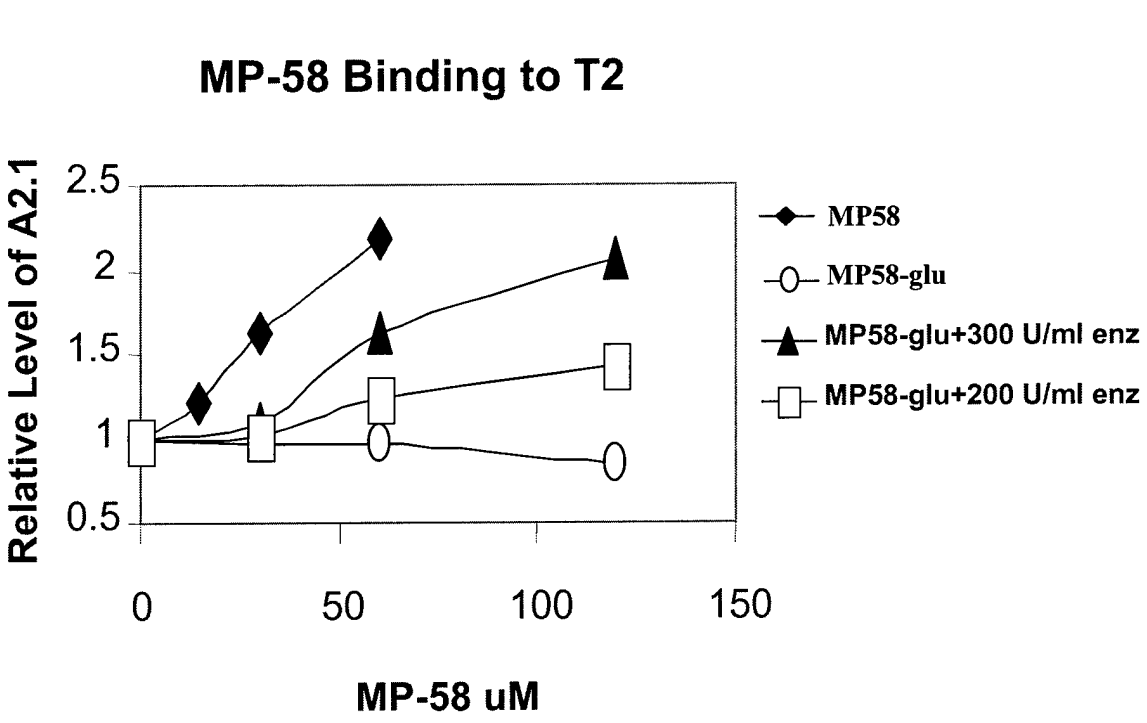


(B) Measurement of peptide and pro-peptide binding to MHC

The binding of peptide or pro-peptide to HLA-A2.1 was measured with T2 cells which are human cells defective in MHC I associated peptide processing. Because of the defect, HLA-A2.1 on these cells do not contain endogenous peptides, are unstable and promptly degraded, resulting in low level of HLA-A2.1 on the surface. Binding of exogenous peptides to A2.1 results in stable MHC/peptide complexes and increased level of surface HLA-A2.1 which can be detected by flow cytometry.

To measure the binding of MP58 peptide or pro-peptide, 3 x 10^5 T2 cells were incubated with varying concentrations of the peptide or pro-peptide. To activate the pro-peptide, beta-glucuronidase (Sigma) was added at 200 or 300 unit/ml to the cell and peptide culture. The samples were incubated at 37°C for one hr before transferred to 28°C for overnight incubation. To measure the level of HLA-A2.1, cells were stained with mAb BB7.2 which recognized human HLA-A2.1 (American Type Culture Collection). Unbound antibody was removed by extensive washing and bound antibody was visualized by staining with FITC conjugated goat anti-mouse IgG. Isotype matched monoclonal antibody was the negative control. Flow cytometric analysis was performed with a FACS Calibur (Becton Dickinson, San Jose, CA) and the results were recorded as mean channel fluorescence.

MP58 peptide bound to T2 cells in a dose dependent manner in the range of 15 to 60 μM. At 60 μM of MP58, HLA-A2.1 level increased to 230% that of control cells which were incubated without peptide nor beta-glucuronidase. The enzyme alone did not affect the level of HLA-A2.1 (not shown). Incubation with MP58 pro-peptide up to 120 μM without glucuronidase had no effect on HLA-A2.1 level, indicating a complete absence of binding by the pro-peptide. In the presence of 200 or 300 unit/ml of beta-glucuronidase, HLA-A2.1 level increased with increasing concentration of the pro-peptide. At 300 unit/ml of the enzyme and 120 mM of MP58 pro-peptide, HLA-A2.1 level increased to 210% of the control value. The same results were obtained in two repeated experiments. Therefore, beta-glucuronidase liberated acti e MP58 from the pro-peptide which bound to HLA-A2.1 as did the native peptide. The cells and peptide were incubated with beta-glucuronidase at near neutral pH to approach the physiological condition. Although not optimal for the activity of beta-glucuronidase, there is significant release of active peptide to mark the cells.



KEY RESEARCH ACCOMPLISHMENTS

1. In the first year, an in vitro 3-D tumor growth inhibition assay was established to measure anti-tumor activity of peptide specific cytotoxic T cells
2. In the second year, pro-peptide of flu MP-58 GILGFVFTL was synthesized and purified to 76% purity which was adequate for MHC I binding analysis.
3. As predicted by our design, MP-58 pro-peptide did not bind HLA-A2.1. Upon interaction with β- glucuronidase, active MP-58 was released and bound to HLA-A2.1.

REPORTABLE OUTCOMES

Rawale, S.V., Hryhorczuk, LM., Wei, WZ., Zemlicka, J., Synthesis and preliminary biological investigation of T cell antigenic peptide prodrug activated by β-glucuronidase. 222nd National meeting of the American Chemical Society, August 26-30, 2001, Chicago, Illinois.

CONCLUSIONS

The synthesis and purification of MP58 pro-peptide demonstrated the chemical feasibility of pro-peptide development. The release of active peptide by beta-glucuronidase demonstrated the precise and controlled generation of an antigenic epitope from a stealth agent as predicted by our design. MP58 pro-peptide is a new and novel agent for cancer immunotherapy.

APPENDIX

Rawale, S.V., Hryhorczuk, LM., Wei, WZ., Zemlicka, J., Synthesis and preliminary biological investigation of T cell antigenic peptide prodrug activated by β-glucuronidase. 222nd National meeting of the American Chemical Society, August 26-30, 2001, Chicago, Illinois.

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Synthesis and preliminary biological studies of T-cell antigenic peptide prodrug activated by β-glucuronidase

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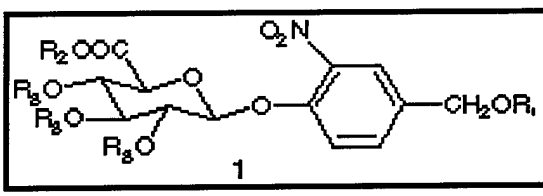
Synthesis of antigenic peptide prodrug 1d is described. The known β-glucuronate glycoside 1a was attached to a glycine moiety via a urethane linker to give /after deacetylation and conversion to pentafluorophenyl (Pfp) ester/ compound 1b. The latter was reacted with octapeptide ILGFVFTL prepared by a solution synthesis to give 1c. Hydrolysis of ester afforded the target prodrug 1d. β-Glucuronidase cleavage furnished nonapeptide GILGFVFTL. Preliminary results with T2 cells will also be reported. Supported by DOD grant BC980459.



1a: R_1 = H, R_2 = Me, R_3 = Ac
1b: R_1 = OCO-GlyPfp, R_2 = Me, R_3 = H
1c: R_1 = OCO-GILGFVFTL, R_2 = Me, R_3 = H
1d: R_1 = OCO-GILGFVFTL, R_2 = R_3 = H

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